PERGAMON

Chemosphere 44 (2001) 1201–1211

CHEMOSPHERE

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Kinetics of thermophilic anaerobes in fixed-bed reactors

M. Perez *, L.I. Romero, D. Sales

Chemical Engineering, Food Technology and Environmental Technology Department, Faculty of Sea Sciences, University of Cadiz, Campus Rio San Pedro s/n, Apdo. 40, 11510 Puerto Real, Cadiz, Spain

Received 3 March 2000; received in revised form 15 June 2000; accepted 22 June 2000

Abstract

The main objective of this study is to estimate growth kinetic constants and the concentration of "active" attached biomass in two anaerobic thermophilic reactors which contain different initial sizes of immobilized anaerobic mixed cultures and decompose distillery wastewater. This paper studies the substrate decomposition in two lab-scale fixed-bed reactors operating at batch conditions with corrugated tubes as support media. It can be demonstrated that high microorganisms-substrate ratios favor the degradation activity of the different anaerobic cultures, allowing the stable operation without lag-phases and giving better quality in effluent. The kinetic parameters obtained – maximum specific growth rates (μ_{max}), non-biodegradable substrate (S_{NB}) and "active or viable biomass" concentrations (X_{V_0}) – were obtained by applying the Romero kinetic model [L.I. Romero, 1991. Desarrollo de un modelo matemático general para los procesos fermentativos, Cinética de la degradación anaerobia, Ph.D. Thesis, University of Cádiz (Spain), Serv. Pub. Univ. Cádiz], with COD as substrate and methane (CH_4) as the main product of the anaerobic process. This method is suitable to calculate and to differentiate the main kinetic parameters of both the total anaerobic mixed culture and the methanogenic population. Comparison of experimental measured concentration of volatile attached solids (VS_{att}) in both reactors with the estimated "active" biomass concentrations obtained by applying Romero kinetic model [L.I. Romero, 1991. Desarrollo de un modelo matemático general para los procesos fermentativos, Cinética de la degradación anaerobia, Ph.D. Thesis, University of Cádiz (Spain), Serv. Pub. Univ. Cádiz] shows that a large amount of inert matter is present in the fixed-bed reactor.

Keywords: Anaerobic thermophilic digestion; Fixed-bed reactor; Biokinetic; Active biomass; Industrial wastewater treatment; Thermophiles

1. Introduction

Anaerobic treatment of wastewater is a possibility to reduce water pollution, which receives nowadays more and more attention. In this process, wastewater is purified by anaerobic bacteria which convert the organic pollutants into methane, carbon dioxide, and new biomass. Since growth rates of anaerobic bacteria are very low, a long retention time in an anaerobic reactor is required to reach the adequate degradation level of organic matter. Therefore much research has been directed to the development of techniques for maintaining a high concentration of biomass in anaerobic wastewater treatment: upflow anaerobic sludge blanket (UASB) (Lettinga et al., 1991; Jhung and Choi, 1995), upflow and downflow stationary packed beds (Kennedy et al., 1987; Young and Yang, 1989; Nebot et al., 1995), and fluidized and expanded beds (Switzenbaum, 1983; Jewell, 1987; Iza, 1991; Pérez et al., 1996b).

In the mixed culture of an anaerobic reactor, several different degradation reactions take place. Complex kinetic, interactions and different steps have been reported by numerous authors (McCarty and Smith, 1986). Soluble substrates can be divided into three major stages: acidogenesis, acetogenesis and methanogenesis. In the

* Corresponding author. Tel.: +34-95601-6158; fax: +34-95601-6040.

E-mail address: montserrat.perez@uca.es (M. Perez).

0045-6535/01/$ - see front matter
PII: S0045-6535(00)00264-2

first combined step, compounds, e.g. carbohydrates are hydrolyzed by extracellular enzymes to organic monomers, e.g. glucose; glucose is later used by the acidogenic bacteria as a substrate and is converted to organic acids, mainly acetic, propionic and butyric. This first step is faster than the subsequent reactions, where the higher acids are all converted to acetic acid. The final product, a biogas containing methane and carbon dioxide, is produced by methanogens along two different pathways. The acetoclastic path producing approximately 72% of methane (Denac et al., 1988) and all the rest is produced by the utilizing H_2 bacteria.

The biomass retention capacity of a reactor, the specific sludge activity, the substrate biodegradability and concentration, and the daily flow-rate availability of the wastewater will define the performance of the process. The loading capacity and the subsequent biogas production rate and wastewater depollution yield are dictated by the amount of "active" biomass available to grow on the biodegradable fraction. In order to evaluate the treatment efficiency and to research the fermentation kinetics in an attached growth reactor such as anaerobic fixed-bed reactor, biomass concentration is basically the necessary information. Although some researchers measure volatile solids (VS) as an index of attached biomass, the index also accounts for inert organic matter like solid metabolites and inviable biomass so that is not directly related to degradation activity. In other words, "active"or viable biomass concentration is more essential to evaluate treatment efficiency. In spite of its importance, the "active"biomass concentration has been reported in very few papers which deal with attached biomass because it is very difficult to measure (Kuba et al., 1990).

The purposes of this study are: (i) to evaluate the attached biomass concentrations (VS_{att}) in the reactors; (ii) to elucidate the treatment efficiency in thermophilic anaerobic fixed-bed reactors which decompose distillery wastewater and (iii) to estimate kinetic constants and "active" biomass concentration using an autocatalytic kinetic model (Romero, 1991) and to compare the measured concentrations of attached biomass (VS_{att}) with the "estimated active" biomass concentrations.

2. Kinetic model

Romero (1991) propose a general kinetic model to describe the performance of degradation processes at different experimental conditions.

2.1. Foundations of the model

The set of biochemical reactions constituting the metabolic route by which the micro-organisms growth can be simplified to the following scheme:

$$X_V + S_B \xrightarrow{k} \Gamma X_V + \cdots \quad (\Gamma > 1), \tag{1}$$

where k represents the rate constant of the process, S_B the concentration of biodegradable substrate, X_V the concentration of viable or active micro-organisms and Γ is a stoichiometric constant. Eq. (1) assumes that the overall metabolic pathway can be simplified by considering that all the reactions giving rise to the total transformation are connected in series and that one of them is the rate controlling stage.

Eq. (1), that illustrates an autocatalytic process because the active micro-organisms (X_V) reproduce, so that, during the process, there is a net generation of such micro-organisms. If the formation of products follows a growth-related mechanism, their formation kinetics can be described, including in the second term of Eq. (1) the concentration of products formed. Other mechanisms require the inclusion of another equation to express the product formation kinetics.

Finally, if it is accepted that the consumption of substrate for maintaining the population is negligible in comparison with the consumption for growth, Eq. (1) is representative of the process as a whole.

2.2. General equation of the model

In this case, on the basis of the proposed reaction scheme, and accepting that growth rate depends linearly on the concentration of both reactants, the following is obtained:

$$\begin{aligned}
(r_X) &= \left(\frac{dX_V}{dt}\right) = (\Gamma - 1) k S_B X_V, \\
(-r_S) &= \left(-\frac{dS_B}{dt}\right) = k S_B X_V, \\
Y_{X/S} &= \left(\frac{dX_V}{-dS_B}\right) = (\Gamma - 1).
\end{aligned} \tag{2}$$

Given the previous definition of the biomass substrate yield coefficient

$$\begin{aligned}
\left(\frac{dX_V}{dt}\right) &= Y_{X/S}\left(-\frac{dS_B}{dt}\right) = Y_{X/S}\left(-\frac{dS_T}{dt}\right), \\
X_V &= X_{V_0} + Y_{X/S}(S_{B_0} - S_B) \\
&= X_{V_0} + Y_{X/S}(S_{T_0} - S_T).
\end{aligned} \tag{3}$$

Last expression for X_V includes the variable S_T which is easily measurable than S_B . Likewise, relationship between both S_B and S_T is

$$S_T = S_B + S_{NB}, \tag{4}$$

where S_{NB} represents the concentration of non-biodegradable substrate by the micro-organisms responsible for the process under examination.

Variables of greater significance are used for the wastewater treatment processes, such as the specific rates

$$\begin{aligned}\mu &= \left(\frac{1}{X_V}\right)\#\left(\frac{dX_V}{dt}\right) = Y_{X/S}kS_B = Y_{X/S}k(S_T - S_{NB}),\\ (-q_S) &= \left(\frac{1}{X_V}\right)\left(-\frac{dS_B}{dt}\right) = kS_B = k(S_T - S_{NB}).\end{aligned} \tag{5}$$

According to Eq. (4), both expressions are linear with the concentration of useful substrate present in the medium, and their maximum values are as follows:

$$\begin{aligned}\mu_{max} &= Y_{X/S}(-q_S)_{max} = Y_{X/S}k(S_B)_{max}\\ &= Y_{X/S}k(S_{T_0} - S_{NB}),\\ k &= \frac{\mu_{max}}{Y_{X/S}(S_{T_0} - S_{NB})}.\end{aligned} \tag{6}$$

Eqs. (1), (2), (3) and (5) give the general equation for the proposed model

$$\begin{aligned}(-r_S) &= kS_BX_V\\ &= \frac{\mu_{max}S_BX_V}{Y_{X/S}(S_{T_0} - S_{NB})}\\ &= \frac{\mu_{max}\left(X_{V_0}/Y_{X/S} + S_{T_0} - S_T\right)(S_T - S_{NB})}{(S_{T_0} - S_{NB})}.\end{aligned} \tag{7}$$

Last expression makes clear the effect of the initial micro-organism's concentration on the substrate utilization rate.

Defining constant "h" incorporating all the initial values of the variables in the previous expressions

$$h = \frac{X_{V_0}}{Y_{X/S}} + S_{T_0}. \tag{8}$$

The constant h has substrate concentration dimensions and represents its maximum value which could be used to generate biomass. It will be seen that this constant is equal to the sum of the concentration of substrate fed to the medium and the factor $X_{V_0}/Y_{X/S}$. This last factor represents the contribution of the concentration of substrate used in the formation of biomass in the medium prior to the addition of the alimentation (inoculum in discontinuous processes).

From Eqs. (3) and (8), it is deduced that

$$\begin{aligned}&X_V = Y_{X/S}(h - S_T),\\ &X_{V_{max}} = Y_{X/S}(h - S_{NB}),\\ &(h - S_T) = \frac{X_V}{Y_{X/S}},\\ &(h - S_{NB}) = \frac{X_{V_{max}}}{Y_{X/S}}.\end{aligned} \tag{9}$$

Considering the expression for "h" constant, defined in Eq. (9), the general model equation is as follows:

$$(-r_S) = \mu_{max}\frac{(h - S_T)(S_T - S_{NB})}{S_{T_0} - S_{NB}}. \tag{10}$$

2.3. Simplifications of the proposed model

From knowledge of the physical significance of the parameters of the model, it is deduced that a variety of simplifications are possible:

(a) When all the substrate fed into the system are useable or biodegradable $S_{NB} = 0$ and, in this case, the general expression obtained is reduced to give the following:

$$\begin{aligned}&S_{NB} = 0 \;\Rightarrow\; S_T = S_B,\\ (-r_S) &= \frac{\mu_{max}}{Y_{X/S}S_{T_0}}X_VS_T\\ &= \frac{\mu_{max}}{S_{T_0}}\left[-S_T^2 + hS_T\right].\end{aligned} \tag{11}$$

(b) When the initial concentration of micro-organisms present in the medium is high enough not to produce an appreciable variation in concentration during the process (as occurs with systems with internal biomass retention), the factor $(h - S_T)$ remains virtually constant so that

$$\begin{aligned}h - S_T &= \frac{X_{V_0}}{Y_{X/S}} + S_{T_0} - S_T\\ &\approx \frac{X_{V_0}}{Y_{X/S}} \;\Rightarrow\; X_V \approx X_{V_0}\\ (-r_S) &= \frac{\mu_{max}}{Y_{X/S}(S_{T_0} - S_{NB})}S_BX_V\\ &\approx \frac{\mu_{max}}{Y_{X/S}}\frac{X_{V_0}}{S_{T_0} - S_{NB}}(S_T - S_{NB}).\end{aligned} \tag{12}$$

Taking account of the significance of parameter "h" (Eq. 8), this condition is met when

$$X_{V_0} \geqslant Y_{X/S} \times (S_{T_0} - S_{NB}) = Y_{X/S} \times (S_{B_0}),$$

in other words, when the biomass concentration in the inoculum is greater than or equal to the maximum which can be formed during the course of the process as such. Naturally, the greater X_{V_0}, the more the representation will approximate to a straight line. The point of intersection of all the curves with the x-axis represents the value of the non-biodegradable substrate concentration in the supply.

(c) Likewise, when, X_{V_0} is sufficiently high, there is no concentration of non-biodegradable substrate

$$\begin{aligned}&S_{NB} = 0 \;\Rightarrow\; S_T = S_B,\\ &(-r_S) = \frac{\mu_{max}}{Y_{X/S}}\frac{X_{V_0}}{S_{T_0}}S_T.\end{aligned} \tag{13}$$

The development kinetic model presents four parameters with different physical and microbiological signifi-

cance: S_{T_0}, S_{NB}, h and μ_{max}. S_{T_0} is the initial substrate concentration (as COD); S_{NB} represents the non-biodegradable concentration of substrate by the micro-organisms; h represents the maximum biomass concentration value reached in the reactor when all the biodegradable substrate have been metabolized by the micro-organisms (expressed as COD units through the yield coefficient $Y_{X/S}$). This constant is equal to the addition of the initial fed substrate concentration (S_{T_0}) and the factor $X_{V_0}/Y_{X/S}$, being the last factor the contribution of the substrate concentration used to generate biomass in the medium prior to the addition of the initial substrate (that is to say, inoculum or attached biomass in discontinuous processes); μ_{max} is the maximum specific growth rate of the micro-organisms responsible for the biodegradation process.

With the assumption that the methane production rate is a linear function of the substrate utilization rate in the filter bed volume, the methane production rate in a batch system can be written as

$$\frac{\partial CH_4}{\partial t} = K\left(-\frac{\partial S_T}{\partial t}\right), \tag{14}$$

$$CH_4 = (CH_4)_0 + K(S_{T_0} - S_T) = K(S_{T_0} - S_T). \tag{15}$$

Substituting Eqs. (10) and (12) into Eq. (15), the following equations can be obtained:

General model:

$$CH_4 = K\left(\frac{\exp(\phi t) - 1}{(1/(h - S_{T_0})) + (\exp(\phi t)/(S_{T_0} - S_{NB}))}\right). \tag{16}$$

Simplified model:

$$CH_4 = K(S_{T0} - S_{NB})[1 - \exp(-\phi t)], \tag{17}$$

where ϕ is

$$\phi = \mu_{max}\frac{h - S_{NB}}{S_{T_0} - S_{NB}}. \tag{18}$$

In these cases, the kinetic parameters are in accordance with the physical and microbiological significance of the methanogenic population in the medium. S_{T_0} is the initial substrate concentration (as COD); S_{NB} represents the concentration of substrate which could not be used to generate methane by the methanogenic micro-organisms. The $X_{V_0}/Y_{X/S}$ parameter represents the initial "active" methanogenic micro-organisms concentration; μ_{max} is the maximum specific growth rate of the methanogenic culture and K is the yield coefficient for methane production expressed as the ratio between methane produced to substrate used for that.

3. Materials and methods

This paper relates the thermophilic anaerobic biodegradation of vinasses and the kinetic constants of the process in anaerobic fixed-bed reactors which contain different sizes of attached anaerobic biomass. The anaerobic filters (AF) were operated as batch lab-scale reactors with corrugated tubes as a support media at thermophilic conditions (55°C).

3.1. Experimental reactor

A process flow schematic of the anaerobic filter reactor is shown in Fig. 1. The reactor was built as a vertical cylindrical tank (25 cm length and 10 cm internal diameter). The active liquid volume was 2 l, and the empty volume was 2.4 l. The reactor was filled with 600 randomly distributed media support entities (16 mm length every one). Reactor temperature was maintained at 55°C. Effluent recirculation was used to mix and homogenize the liquid in the system (recycle rate: 6 l/h). In these conditions, the liquid phase is perfectly mixed (tracer studies corroborate this affirmation). Gas produced in the reactor was collected in a gas-meter filled with acidified saturated salt solution. A gas sampling valve was installed at the top of the collector to allow direct gas sampling with a syringe. The volume of produced gas was directly measured in terms of the volume of salt solution displaced from the gas collector.

3.2. Feed solutions

Distillery wastewater from an ethanol producing wine-distillery plant placed in Tomelloso (Ciudad Real, Spain) was used. In general, vinasses show an adequate relationship between the different macro- and micro-

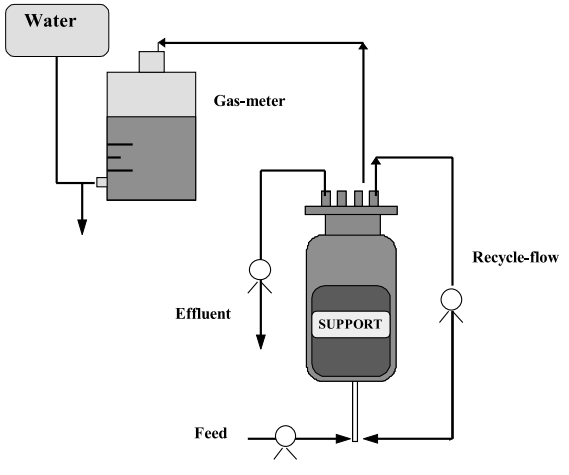

Fig. 1. Schematic diagram of the experimental reactor.

nutrients with a COD/N/P ratio suitable for microbiological treatment. The vinasses were frozen and then transported and maintained at 4°C before their utilization. This feed (30 g COD/l) was diluted with tap water to attain the required feed chemical oxygen demand (COD) concentration to be used in these experiments (15 g COD/l) and was supplemented with sodium hydroxide (NaOH 7N) to adjust a neutral pH value.

After this study, batch experiments (Pérez et al., 1996a) were conducted in order to investigate the biodegradability of the vinasses by the anaerobic biomass. The results obtained indicate that this feed was a complex medium formed by two substrates with different nature and biodegradability: S_1, the easily biodegradable substrate fraction (80% of the total), and S_2, the non-easily biodegradable substrate fraction (recalcitrant substrate) in the conditions that the experiments were conducted.

3.3. Support media

Corrugated plastic tubes (non-porous media) were used as support media for cell immobilization and retention in this research. This support offers low density (1161.4 g/l), high porosity (93%) and high specific area (450 m^2/m^3) suitable for use as packed media in anaerobic reactors. Others characteristics of the support are: apparent density: 73.0 g/l, height and diameter: 1.6 cm. Fig. 2 shows electronic microscopy micrographs of plastic support: (a) originally, non-populated material; (b) detail of populated particle surface; (c) populated support with magnification 12.000; (d) magnification 22.000 (Nebot, 1992).

3.4. Analytical methods

All analytical determinations were performed according to Standard Methods (APHA, 1989). For liquid samples, the analyzed parameters in both the effluent and the influent were: pH, COD, both total and volatile suspended solids (TSS, VSS) and attached microbial mass (VS_{att}). For gaseous samples, the parameters analyzed were the volume of biogas produced at STP conditions and its composition.

COD was determined by the dichromate reflux methods. For soluble CODs, the sample was first filtered as in the TSS analysis and the filtrate was used for the COD analysis. TSS and VSS were determined by the glass fibber filter method as described in Standard Methods. Determinations of methane and carbon dioxide were carried out by gas chromatography separation with a stainless steel column packed with Carbosive SII (diameter of 1/8 in. and 2 m length) and thermal conductivity detector (TCD). The injected sample

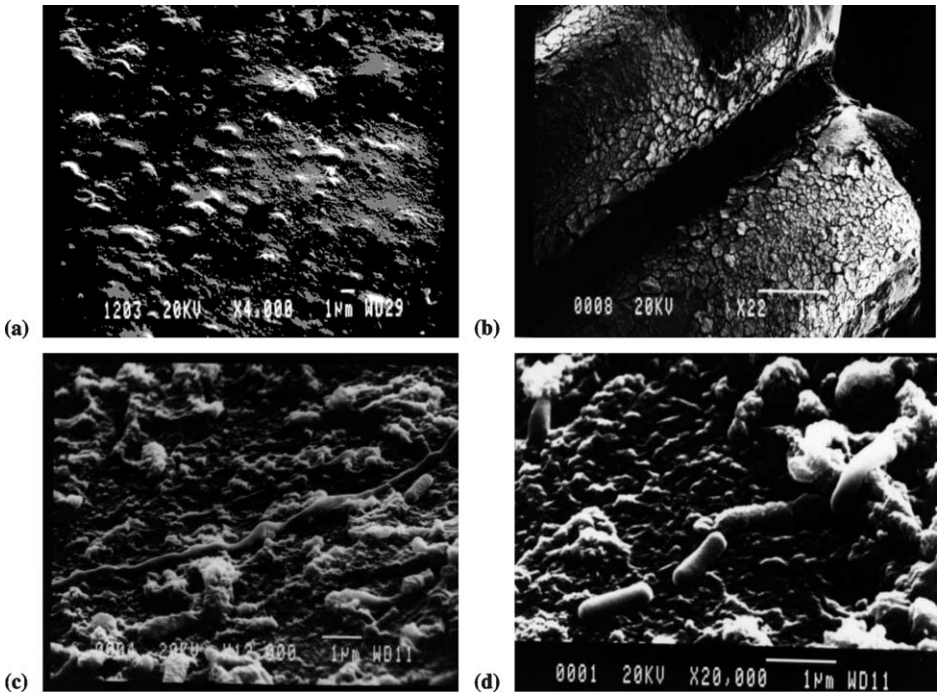

Fig. 2. Electronic microscopy micrographs of plastic support: (a) originally, non-populated material; (b) detail of populated particle surface; (c) populated support with magnification 12.000; (d) populated support with magnification 22.000 (Nebot, 1992).

volume was 1 cm^3 and operational conditions were as follows: 7 min at 55°C; rammed at 27°C/min until 150°C; detector temperature: 255°C; injector temperature: 100°C. The carrier was helium and the flow rate used was 30 ml/min. A standard gas (by Carburos Metálicos, S.A; composition: 4.65%H_2; 5.33%N_2; 69.92%CH_4 and 20.10%CO_2) was used for the calibration of the system.

Before operating each system, the immobilized biomass density on the plastic support was evaluated. VS_{att} concentrations were determined by removing a representative sample from the reactor (20 units were extracted randomly from four different fractions, considering a total of 600 support units in the total bed volume). The adhered biomass was separated from the support by applying a high pressure waterjet (at 55°C) up to a total volume of 250 ml. Both, VSS and TSS concentrations of these watery solutions were determined.

4. Results and discussion

4.1. Attached biomass

Initially, 20 units of biocovered media samples were taken from four different fractions of the reactors in order to measure VS_{att} concentrations as an indication of biomass concentration in each fraction (numbered from top to bottom). Fig. 3 illustrates the profiles of attached biomass, expressed as g VS_{att} in each fraction of each reactor. Table 1 shows the quantification of TS and VS in four fractions considered in FA1 and FA2 reactors.

The AF2 filter contains 9.81 g VS_{att}/l digester, approximately twice more than AF1 (5.25 g VS_{att}/l digester). As can be seen in Fig. 3, AF1 presents the same VS_{att} density in all the studied fractions. However, AF2 reactor presents a profile of biomass with a maximum in the bottom of the reactor. Nebot (1992), in analogous studies of characterization of biomass density in down-flow fixed filter reactor, remarks a similar evolution of both VS_{att} and biomass activity (as mg COD/g VS_{att} l d). These are higher in upper fractions. The author attributes this effect to the substrate transfer limitations as well as inhibition effects by the precipitate salts.

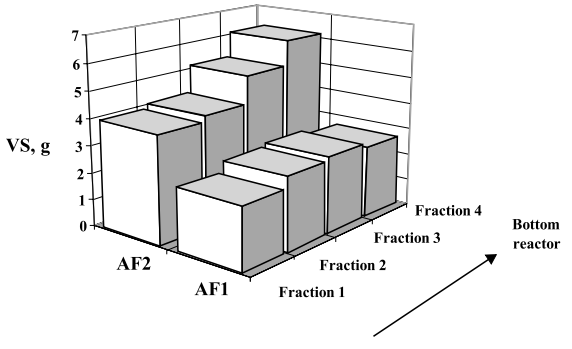

Fig. 3. Representation of biomass density in different fractions of the anaerobic fixed-bed reactors.

Table 1
Quantification of TS and VS in the four different fractions of FA1 and FA2

	ST (%)	SV (%)	SV/ST
FA1			
Fraction 1	21.57	21.88	1.01
Fraction 2	26.39	25.69	0.97
Fraction 3	26.81	27.02	1.01
Fraction 4	25.31	25.31	1.00
Total (g)	12.02	10.51	0.87
FA2			
Fraction 1	22.71	20.39	0.88
Fraction 2	21.53	21.30	0.99
Fraction 3	24.53	26.71	1.09
Fraction 4	31.18	31.65	1.01
Total (g)	21.97	19.62	0.89

4.2. Anaerobic degradation

4.2.1. Evolution of substrate

The COD removal was different for AF1 and AF2. As can be seen in Fig. 4(a), AF1 presents a short lag-phase and approximately at 46 h, the substrate consumption is only 20% COD. In this first step of the process, the pH decreases below 7 indicating an increase in total organic acid concentration. After this phase, when the pH is held through 7, a sudden drop of substrate is observed and, finally, a COD stabilization phase is reached (2.7 g COD/l) reaching a depurative efficiency of 82% COD in 518 h. The pH remains constant about 8.5.

On the other hand, the higher VS_{att} in AF2 implicates an accelerate biodegradation process without lag-phase. The substrate concentration decreased almost linearly with time. The linear decrease indicated that increases of biomass associated with the substrate decomposition was negligible to initial biomass concentration (Kuba et al., 1990). The total residual substrate is 1 g COD/l approximately corresponding to a removal efficiency of 93% COD, upper than AF1. The pH is held near to neutral values in all stages of the process.

To our knowledge, this behavior is due to the different VS_{att} concentrations in each reactor: hence, the higher concentration improves the development of the different bacterial groups implicated in the process and the stability of the methanogens without lag-phases. Additionally, the presence of enough micro-organism

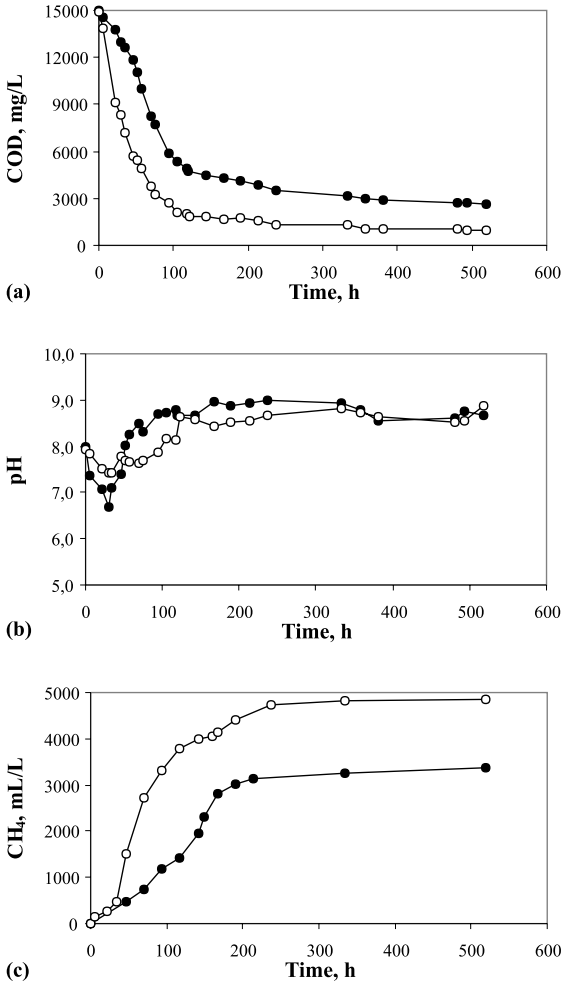

Fig. 4. Experimental data evolution curves of: (a) substrate concentration (expressed as mg COD/l digester); (b) pH; (c) methane (expressed as ml CH_4/l digester), versus time (h) (•: AF1, ○: AF2).

densities (both acidogenics and methanogenics) implicate the simultaneous degradation of S_2 and S_1 substrates, even when S_2 has been defined as a recalcitrant substrate.

In conclusion, a higher micro-organisms: substrate ratio can reduce and even eliminate the lag-phase and facilitate the metabolization of recalcitrant substrates. Hence, the non-biodegradable COD mass fraction in the medium increases with the decreasing of VS_{att} concentration in the reactors.

4.2.2. Evolution of biogas

Both reactors, AF1 and AF2, generate the same biogas volume (6 l approx.) but different methane volume in the incubation period (519 h). This evolution of methane generation in both reactors is shown in Fig. 4(c). The AF1 reactor presents an initial lag-phase, with 2 l CH_4 in 143 h opposite AF2, with 4 l CH_4 in the same time.

The accumulated methane volume in AF1 is 3.4 l CH_4 (63.8% of the total biogas), and represents a yield of 0.27 l CH_4/g COD_r. This value is lower than the stoichiometric theoretical of 0.35 l CH_4/g COD_r (1 g of COD is equivalent to 0.35 l of methane at STP). Since the initial biomass in AF1 was lower, the increment of biomass (synthesis of new micro-organisms), therefore, must be considered in this reactor. This phase also implies a high consumption of organic material for the synthesis route (anabolism), diminishing, therefore, the substrate amount which is transformed into methane. In this sense, the theoretical value of 0.35 l CH_4/g COD_r decreases the calculated experimental value. The AF2 reactor generates 4.8 l CH_4 (82% of total biogas) with a yield of 0.35 l CH_4/g COD_r (coinciding with the theoretical value).

4.3. Process modelization

Romero kinetic model (Romero, 1991) was used to evaluate the kinetic parameters and "active biomass" in the processes.

Parameters values of the model (expressions (10) and (12)) are representative of the micro-organisms responsible for the process when a unique population is active with respect to the transformation taken place. In other cases (i.e., when cultives mixed are present), the values of the model parameters consider all the populations. Thus, in anaerobic digestion of complex wastes (i.e. wine vinasses degradation) depending on the state variable fitted to the model equation, the values obtained for the parameters will be representative from the acidogenic population or from the methanogenic ones or from the overall.

In general, microbiological pathways for substrate utilization by micro-organisms include two main routes: anabolic and catabolic pathways. Catabolism is the energy obtention route and in the anaerobic processes is related to the main product formation (oxidative phosphorilation of the substrate). On the other hand, anabolism corresponds to the microbial growth route and it is related to the biomass increase in the medium. However, for fermentative processes in general, other substrate utilization routes must be considered: the maintenance route and the complex product synthesis route. The former is related with the utilization of energy from catabolic pathway to maintain the micro-organisms population in active state, but in anaerobic processes this quantity is accounted through the main product formation in catabolic route. The last route is related with the formation of complex products by the micro-organisms, and in biofilm systems includes the

substrate utilization to the matrix formation (i.e., polysaccharides).

This fact provokes that values of "h", "μ_{max}" and S_{NB} obtained in this study will be depending on the state variable selected to characterize the system performance and to be fitted. When the COD concentration is used, parameter values correspond to both the acidogenic and the methanogenic populations, because all of them are involved in the substrate utilization. However, since the products resulting from the acidogenic population activity are short chain organic acids and new micro-organisms, the COD removed for that will be related to micro-organisms growth and to complex product synthesis, only. On the other hand, COD reduction by methanogenic population will be related to both pathways: organic acids transformation into methane and methanogenic population growth and attachment to the biofilm. On the other hand, when methane production is used as state variable, the model parameter values determined are representative from the methanogenic population only. In this case the S_{NB} value obtained includes, also, COD concentration used for micro-organisms growth and adhesion to media support, and both the X_{V_0} and the μ_{max} correspond to the active methanogenic population.

The changes of the substrate concentration and methane generation with time are expressed according to Romero kinetic model (Romero, 1991). The parameters of the model are estimated by the curve fitting method by non-linear regression of the data using Statgraphics 6.0, based in the Marquardt alghoritm (Marquardt, 1963).

4.3.1. Substrate utilization model

The changes of substrate with time in AF1 can be fitted by the general kinetic model, Eq. (10), which can be integrated to yield

$$S_T = \frac{h(S_{T_0} - S_{NB}) + S_{NB}(h - S_{T_0}) \exp\left(\mu_{max} \frac{h - S_{NB}}{S_{T_0} - S_{NB}} t\right)}{(S_{T_0} - S_{NB}) + (h - S_{T_0}) \exp\left(\mu_{max} \frac{h - S_{NB}}{S_{T_0} - S_{NB}} t\right)}. \quad (19)$$

Firstly, due to the high biomass concentration in AF2 (calculated as VS_{att}) and the experimental curve of evolution of substrate (falling exponential), we accept that AF2 can be fitted by the simplified expression of the model. Simplified kinetic model, Eq. (12) can be integrated to yield

$$S_T = S_{NB} + (S_{T_0} - S_{NB}) \exp\left(\mu_{max} \frac{h - S_{NB}}{S_{T_0} - S_{NB}} t\right). \quad (20)$$

The results of the curve fitting method obtained are shown in Tables 2 and 3. Fig. 5 illustrates both the changes of substrate concentration with the time and the estimated evolution curves. All correlation indexes are through 0.99, indicating the goodness of the fittings. The physical and microbiological significance kinetic parameters permit to compare their values with the experimental results.

Hence, the S_{NB} parameter is closely to the experimental residual COD in each experiment. The value of the maximum specific growth rate, μ_{max}, is in the range between 0.0253 h^{-1} (AF1) and 0.0303 h^{-1} (AF2). As the anaerobic process consists of several different degradation reactions connected in series, the rate of the process is determined by the limiting rate stage and that is considered to be the methanogenic step (which is slow compared with all the others reactions). In balanced processes, the activity corresponds to the simultaneous sequence of the all anaerobic cultures implicates in the process. Furthermore, the bibliographic data indicate that this value of μ_{max} is representative of the maximum specific growth rate of the anaerobic thermophilic micro-organisms. This value is according to the value obtained by Romero et al. (1990), operating with stirred

Table 2
Estimated kinetic constants values according to the substrate consumption model: h (mg COD/l), S_{NB} (mg COD/l) and μ_{max} (h^{-1})

Reactor	S_{T_0}	h	S_{NB}	μ_{max}	r^2
AF1	14 960	16 427	3256	0.0253	0.99
AF2	14 954	27 747	1323	0.0303	0.99

Table 3
Experimental biomass concentrations (VS_{att}, mg VS/digester) and estimated "active" biomass X_{V_0} (mg VS_{att}/digester) by using the substrate consumption model

Reactor	VS_{att}	$X_{V_0}/Y_{X/S}$	X_{V_0}
AF1	10 500	1467	117.4
AF2	19 620	12 793	1023.4

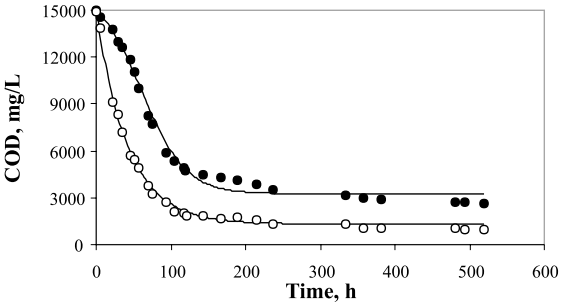

Fig. 5. Experimental data and estimated evolution curves of substrate concentration (expressed as mg COD/l digester) versus time (h) (•: AF1, ○: AF2).

tanks and anaerobic filter technologies in the anaerobic thermophilic of distillery wastewater treatment, as well as the propose by Chen and Hashimoto (1980), from an experimental correlation of μ_{max} as a function of the temperature by the fitting method of the data of a elevate number of authors.

"*h*" estimated values justify a higher biomass concentration in AF2, as has been indicated starting from the experimental results of VS_{att} in each reactor (AF1: 5.25 g VS_{att}/l digester and AF2: 9.81 g VS_{att}/l digester). In both experiments the active micro-organisms concentration at the beginning of the essays must include the attached biomass and the suspended volatile solids. However, the last value is insignificant in the experimental conditions used, and therefore, $X_{V_0}/Y_{X/S}$ is due, fundamentally, to the attached micro-organisms in the support. Assuming a yield coefficient of substrate related to biomass, $Y_{X/S} = 0.08$ kg VS/kg COD (Romero et al., 1988, 1990), we can obtain an "active" biomass concentration (X_{V_0}) of 117 and 1023 mg VS in AF1 and AF2, respectively, which are much lower than the measured values. This fact indicates that high inert organic materials concentration and "non-active" biomass are included into the attached biofilm.

4.3.2. Product generation model

The evolution of the product (methane) with time could be fitted by the general, Eq. (16), and simplified, Eq. (17), expressions of the Romero model, respectively, as the same way as that in the adjustment of experimental data to substrate utilization model.

However, methane evolution with time in AF2 cannot be fitted to simplified expression of the model, as in the previous case, because the initial active methanogenic population responsible to methane formation is not high enough to make neglected the variation in concentration during the process, and the simplified model is not adequate to describe this process. In this sense, we can assume the fit to the general kinetic expression (17).

The results of the fittings obtained are shown in Table 4. The experimental attached biomass concentrations and estimated "active" methanogenic biomass concentrations are listed in Table 5. Fig. 6 illustrates the changes of methane with the time and the estimated evolution curves.

All correlation indexes are through 0.99, indicating the goodness of the fittings. In both cases, the values of the kinetic parameters obtained are according to its physical and microbiological significance.

In both reactors, the S_{NB} parameter values represent the concentration of substrate which could not be used to generate methane by the methanogenic micro-organisms, and they are higher than the values obtained in the fittings to substrate utilization model (more noticeable in AF1). This fact indicates that a portion of substrate is consumed by other routes: synthesis of new micro-organisms (anabolism) and the initial attachment biomass processes on support surface, that suppose a great consumption of organic material.

Table 4
Estimated kinetic constants values according to the methane production model: *h* (mg COD/l), S_{NB} (mg COD/l), μ_{max} (h^{-1}) and *K* (l CH_4/kg COD_r)

Reactor	S_{T_0}	*h*	S_{NB}	μ_{max}	*K*	r^2
AF1	14960	15476	5305	0.0239	0.35	0.98
AF2	14954	17413	1754	0.0243	0.35	0.98

Table 5
Experimental biomass concentrations (VS_{att}, mg/digester) and estimated "active" methanogenic biomass: X_{V_0} (mg VS_{att}/digester) by using the methane production model

Reactor	VS_{att}	$X_{V_0}/Y_{X/S}$	X_{V_0}
AF1	10500	516	41.3
AF2	19620	2459	196.7

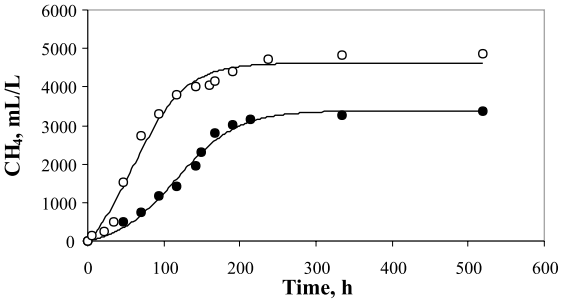

Fig. 6. Experimental data and estimated evolution curves of methane (ml CH_4/l digester) versus time (h) (•: AF1, ○: AF2).

The difference between the values of S_{NB} of both fittings, $(S_{NBT} - S_{NBM})$, represents the substrate used by acidogenic micro-organisms to growth and attachment to solid surface of biofilm. These values are 2049 and 431 mg COD l^{-1}, in AF1 and AF2, respectively. Comparison of those factors with the values obtained for $X_{V_0}/Y_{X/S}$ in Table 3 (kinetic parameters of substrate utilization model) justify that, in AF2, the increase of biomass associated with the substrate decomposition is negligible to initial biomass concentration, which is corroborated with the stability in "*h*"value.

The μ_{max} parameter, approx. 0.024 h^{-1}, is the maximum specific growth rate of the methanogenic culture implicated in the process. This is similar to the value obtained in the previous fitting for substrate consumption of AF1, indicating that in this reactor, the methanogenic is the limiting step. This value is slightly higher than the values proposed by (Kuba et al. (1990)) for the different individual methanogenic populations (HAc, HPr and HBu decomposing bacteria).

Table 6
Comparison between kinetic parameters values S_{NB} (mg COD/l); μ_{max}(h^{-1} and X_{V_0} (mg VS_{att}) estimated by using both the substrate consumption and the methane production models

	Expression	S_{NB}	μ_{max}	X_{V_0}
Substrate utilisation model				
AF1	General	3256	0.0253	117.4
AF2	Simplified	1323	0.0303	1023.4
Methane production model				
AF1	General	5305	0.0239	41.3
AF2	General	1754	0.0243	196.7

The "h" estimated values justify the usefulness of general model in both experiments. These values are lower than the previous values, due in this case to the fact that only "active methanogenic biomass" has been evaluated.

The yield coefficient of product related to biomass, K, is similar to the stoichiometric theoretical of 0.35 l CH_4/g COD_r.

The comparative results of both fittings are written in Table 6. The comparison of the measured concentration of VS_{att} with the estimated "active" biomass concentrations indicate that a large amount of inert matter exists in the fixed-bed reactor.

5. Conclusions

The following conclusions have been drawn from the results of the experiments realized:

1. It can be demonstrated that high micro-organisms-substrate ratios favor the degradation activity of the different anaerobic bacteria populations, which allow to operate in stable conditions without lag-phases and to give better qualities in the effluent discharged.
2. Selected kinetic model (Romero, 1991) can be used to describe the performance of thermophilic anaerobic fixed-bed reactors with respect to both the substrate utilization and the product generation. The values of kinetic parameters of the model permit to differentiate between the main groups of micro-organisms implicated in the process with mixed cultures. Finally, the model is able to estimate the initial active micro-organisms concentration for each of them and to predict the change in the observed kinetic behavior of the system in function of X_{V_0} value.
3. The estimated "active" or viable biomass concentrations are compared with the experimental concentrations of VS_{att} measured in the bed. They are much lower than the measured values. This fact indicates that large amount of inert matter ("non-active" biomass) exist in the fixed-film reactor.

Acknowledgements

This work was supported by grants from the "Comisión Interministerial de Ciencia y Tecnología" (CICYT, BIO 92-0859 – Madrid, SPAIN).

Appendix A

AF	anaerobic fixed-bed reactor
COD	chemical oxygen demand, ML^{-3}
COD_r	chemical oxygen demand removal, ML^{-3}
h	maximum micro-organisms concentration attainable in the medium expressed as biodegradable substrate, ML^{-3}
k	autocatalytic process kinetics constant
K	yield coefficient of product related to substrate for mixed-growth-associated production (dimensionless)
S_B	biodegradable substrate concentration, ML^{-3}
S_{B_0}	initial biodegradable substrate concentration, ML^{-3}
S_{NB}	non-biodegradable substrate concentration, ML^{-3}
S_{NBM}	non-biodegradable methanogenic substrate concentration, ML^{-3}
S_{NBT}	non-biodegradable total substrate concentration, ML^{-3}
S_T	total influent substrate concentration, ML^{-3}
S_{T_0}	initial influent substrate concentration, ML^{-3}
t	time (T)
TS	total solids, ML^{-3}
TSS	total suspended solids, ML^{-3}
VS_{att}	volatile attached solids, ML^{-3}
VSS	volatile suspended solids, ML^{-3}
X_V	active micro-organisms concentration, ML^{-3}
X_{V_0}	active micro-organisms concentration at $t=0$, ML^{-3}
$Y_{X/S}$	true yield coefficient of biomass related to substrate (dimesionless)
Γ	stoichiometric coefficient for micro-organisms formation (dimensionless)
μ_{max}	maximum specific growth rate of micro-organisms, T^{-1}
$(-q_S)$	specific rate of substrate consumption, ML^{-3}
$(-r_S)$	net rate of substrate consumption, ML^{-3} T^{-1}
(r_X)	net rate of micro-organims growth, ML^{-3} T

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